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Research Article

Biochemical and Physical Factors Contributing to Seasonal Variations of Fillet Gaping in Gilthead Seabream (*Sparus aurata*)

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The gaping phenomenon in gilthead seabream (*Sparus aurata*) causes profitability problems for the relevant aquaculture industry. To gain insights into the factors affecting gaping in gilthead seabream, the seasonality of gaping intensity and the relative contribution of postmortem physical and chemical factors to the fillet integrity were investigated. Gilthead seabream of commercial weight (400–600 g) were sampled seasonally. Gaping frequencies were evaluated and related to water holding capacity (WHC), muscle pH, proximate composition, ATP breakdown products (*K*-value), total collagen content, and collagen fractions. With the exception of the *K*-value, all examined physical and chemical parameters were seasonally affected. Moreover, gaping score frequencies were found to be statistically different among seasons. It was evident that during seasons with high water temperatures (summer and autumn), gaps in muscle tissue of gilthead seabream were more pronounced, while a mitigation of gaping intensity appeared during wintertime. ATP breakdown as a measure of chemical freshness, proximate composition, pH, and collagen levels, showed no correlation with gaping intensity, but a clear negative correlation was observed between high gaping incidences and WHC. There was also a strong correlation between gaping incidences and collagen fractions. A higher amount of acid-soluble collagen (ASC) was observed during summer, highlighting that collagen solubility affects gaping in farmed gilthead seabream. Overall, our results demonstrate that seasonal gaping variability in this species is primarily a result of temperature-driven changes in collagen solubility.

Keywords: collagen fractions; gaping; gilthead seabream; pH; season; *Sparus aurata*; water holding capacity

1. Introduction

In Mediterranean marine finfish farming, gilthead seabream (*Sparus aurata*) represents the leading species, and hence a highly commercialized product for European aquaculture [1]. Over the last decades, production of gilthead seabream has experienced an almost thirtyfold increase; it escalated from 10,501 tons in 1993 to 277,435 tons in 2022 (FishStatJ, 2024). This growth stems from advancements in cage farming technology, coupled with the rapidly increasing demand for high-quality aquaculture products with unique gastronomical characteristics, such as those attributed to euryhaline farmed fish species [2].

Gilthead seabream is primarily marketed as whole, gutted, or as fillets to meet contemporary consumer trends. Filleting, whether mechanical or manual, adds value to the product [3]. However, there are obstacles to further enhancement of fillet production, which include the limited shelf-life of fillets and, hence, transport and retailing limitations [4], and also imminent deterioration of textural integrity, such as tissue softening and fillet gaping [5].

Fillet appearance is affected by muscle cellularity, composition, and macrostructure, all of which critically impact final product quality [6]. Gaping, a well-documented postmortem phenomenon first described over four decades ago [7], compromises fillet integrity. It is usually characterized by

torn connective tissue between muscle layers (myofiber-myocommata attachments and between myofibres), creating visible gaps and slits in the fish fillet [8–10]. Elsewhere, gaping has been described as the result of the interaction between the forces pulling the muscle apart and the strength of the tissue [11]. Undoubtedly, this textural deterioration leads to downgrading of the product and considerable profit loss as consumers reject fillets characterized by gaping due to their unattractive appearance.

Multiple factors have been tightly associated with the propensity of fillets to gap, due to increased stress, caused by handling prior to and during slaughter, being the most prominent factor [12, 13]. In fish farming practices, high muscle glycogen due to intensive feeding leads to depleted low postmortem muscle pH, subsequently affecting fillet texture [5, 14, 15]. Seasonal variations in gaping intensity have also been linked to management conditions, including harvest location [16] though no consistent pattern can be inferred with certainty [17, 18]. Textural characteristics are further influenced by chemical factors such as tissue composition [19], collagen content, and cross-linking [20, 21]. Notably, Espe et al. [17] reported a higher percentage of soluble collagen in fillets suffering from gaping, when compared to those with intact tissue. This is possibly due to the result of collagenase activity dysregulation in fillet tissue [22]. Key postmortem factors include temperature during storage [14, 23] and the processing methods [24].

Previous attempts to describe the gaping phenomenon on a seasonal basis lack agreement on the reported patterns [14, 17, 18]. These inconsistencies, as well as the incomplete understanding of the mechanisms underlying fillet gaping, make it difficult to draw reliable conclusions about gaping occurrence in gilthead seabream. In this context, acknowledging the scarcity of studies in regard with the fillet gaping in this species, the present work aimed to describe the seasonal variations of gaping in gilthead seabream and to investigate how postmortem physical and chemical factors, namely water-holding capacity, pH, proximate composition, chemical freshness, collagen content, and collagen solubility, contribute to fillet integrity.

2. Materials and Methods

2.1. Samplings. Gilthead seabream of commercial weight (400–600 g) were provided by Avramar SA (Paiania, Attika, Greece). Sampling was carried out throughout the year, at the beginning and in the middle of each of the four seasons (summer, autumn, winter, and spring), that is, a total of eight sampling points. Water temperatures in sea-cage farms during each of the four seasons are presented in Table 1.

Fish were slaughtered and transferred to the company's processing plant (Trypio Lithari, Attika, Greece) according to standard commercial procedures. Then, they were packed in styrofoam boxes with ice at 0°C–4°C and stored for a short time until passed through a mechanical drum for descaling and machine filleting. The entire process was industrial and fully automated. The fish fillets produced were weighed, packed in ice, and quickly transported to the Hellenic Center for Marine Research (HCMR, Anavyssos, Athens, Greece).

Table 1: Seasonal variations in sea water temperature at sampling locations.

Season	Sea water temperature range (°C)	Mean temperature of sea water (°C)
Summer	23–29	27.2
Autumn	19–22	21.9
Winter	13–15	14.5
Spring	16–20	16.2

2.2. Assessment of Gaping. The degree of gaping in each fillet, expressed as a percentage of the surface area covered by gaps, was determined according to the recently published method applicable to the particular species [25]. Specifically, three assessors trained in the recognition and quantification of the severity of gaping in gilthead seabream fillets, categorized the samples based on a 6-point scale as follows: score 0: Absence/No gaping, score 1: Slight/Subtle gaping (up to five small gaps), score 2: Mild gaping (up to seven small gaps), score 3: Moderate gaping (up to seven large and few small gaps), score 4: Severe gaping (up to seven large and/or many small gaps), score 5: Extreme gaping/nonmarketable fillet (over seven large gaps). A total of 495 samples of gilthead seabream fillets were evaluated for gaping occurrence.

2.3. pH Measurement. The unprocessed fish (10 fish/sampling point) and fillets (10 fillets/sampling point) were measured on-site (processing plant) for muscle pH, before and immediately after processing, respectively. Measurements were taken from above the lateral line in the tail part of the samples (Figure 1) using a hand-held HI99161 pH-meter (Hanna Instruments, USA). The electrode was inserted into a small incision cut through the skin or muscle, depending on the sample (whole fish or fillet). Measurements were carried out in duplicate, and subsequent readings were taken through a new incision located 0.5–1 cm away from the initial one.

2.4. Water Holding Capacity (WHC). Liquid loss was measured by the centrifuging loss in 15 samples per season. Fillet samples (3 g; Figure 1) were chopped into small pieces and placed in a perforated round-bottom tube. The tube was placed in a larger centrifuge tube so that the sample would not be in contact with the released liquid. After centrifugation (20 min, 2000*g*, 4°C), the samples and tubes were weighed again. Thereafter, tubes containing the extracted liquid were placed in an oven at 60°C, and fat loss was determined after dehydration to constant weight. WHC was calculated as the difference between the initial percentage of water in the muscle and the percentage of water released during centrifugation.

2.5. Proximate Composition. Proximate composition analyses of gilthead seabream fillets (20 samples/season; Figure 1) were performed according to the standard AOAC 2005 methods. Specifically, moisture content was determined by drying samples at 105°C to constant weight while ash

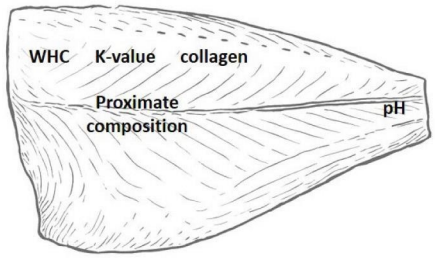

FIGURE 1: Schematic representation of the gilthead seabream fillet indicating the sampling locations used for the analysis of post-mortem factors. Water-holding capacity (WHC), *K*-value, collagen (total content and fractions), proximate composition, and pH were determined at the regions marked on the fillet illustration for each sample.

content was measured after incineration for 16 h at 500°C. Total fat content was weighed after petroleum-ether extraction by Soxhlet. Nitrogen was determined by the Kjeldahl method after acid digestion of samples, and crude protein was subsequently calculated as % nitrogen × 6.25. Glycogen content was calculated by difference and specifically by subtracting the average quantity expressed as a percentage of the other macronutrients from 100.

2.6. Chemical Freshness, K-Value, and ATP Breakdown Products. A high performance liquid chromatography (HPLC) apparatus, combining a Waters 600 Pump, a Waters 717 Plus Autosampler set at 10°C injection temperature, a Waters 2487 UV detector set at 254 and Empower Chromatography Software (Waters, Milford, MA, USA) were used for the analysis of the ATP breakdown products, namely, adenosine-triphosphate (ATP), adenosine-diphosphate (ADP), adenosine-monophosphate (AMP), inosine-monophosphate (IMP), Inosine (Ino), and hypoxanthine (Hx), of 10 unprocessed fish muscle and fillet samples at each time point, according to a previously published methodology [26, 27]. Briefly, 5 g of dorsal muscle tissue (Figure 1) were homogenized on ice with perchloric acid (25 mL, $HClO_4$, 0.6 M) for 2 min. Homogenates were centrifuged (5 min, 4°C at 7000*g*), and 10 mL of the supernatant was transferred to a beaker. Samples were neutralized (pH 6.7–6.9) with the addition of KOH (0.1 and 1 M). Chromatographic separation of ATP breakdown products was achieved using a reverse-phase chromatographic column (C18, 5 μm 100 RP, 4 × 250 mm, Phenomenex, USA);0.4 M KH_2PO_4 and 0.06 M K_2HPO_4 in HPLC water were used for the mobile phase, at a flow rate of 1.5 mL/min. Column temperature was maintained at 30°C, the injected sample volume was 5 μL, and the total run time was 20 min.

Chemical freshness (*K*-value) was calculated as follows:

$$K - \text{value}\% = 100 \times \frac{\text{Ino} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Ino} + \text{Hx}}.$$

2.7. Total Collagen Content. The calculation of total collagen (10 samples/season) was based on the determination of hydroxyproline content. To this end, 0.2 g of muscle tissue (Figure 1) was homogenized in 200 mL of water, and an aliquot (100 μL) was transferred to a pressure-tight polypropylene vial with a PTFE-lined cap. An equal volume of concentrated hydrochloric acid (HCl, 12 M) was added, and the samples were left to hydrolyze in an oven at 120°C for 3 h. Thereafter, hydrolyzed samples were centrifuged at 10,000*g* for 3 min, and an aliquot of the supernatant (40 μL) was transferred to a 96-well plate. Subsequently, the plate was placed in a 60°C oven for moisture to evaporate. Colorimetric determination of hydroxyproline was based on the oxidation of the amino acid with chloramine-T, followed by the addition of 4-dimethylaminobenzaldehyde, resulting in the production of a colored mixture that was measured at 560 nm. A conversion factor of 11.42 was used to convert hydroxyproline into collagen [28], and the triplicate measurements were expressed as mg per g of fillet tissue.

2.8. Soluble Collagen Fraction. Soluble collagen fraction was extracted according to Sato, Yoshinaka, Sato, Itoh, and Shimizu [29]. Specifically, 1.5 g of fillet muscle tissue (10 samples/season; Figure 1) was homogenized in 10 volumes (v/w) of cold 0.1 N NaOH and centrifuged at 10,000*g* for 20 min at 5°C. The supernatant was discarded, and the residue was treated with 20 volumes (v/w) of 0.1 N NaOH. Alkaline extraction was continued under refrigeration (5°C) while being continuously stirred for 16 h. Thereafter, the sample homogenate was centrifuged (10,000 *g*), and the above procedure was repeated in quadruplicate. The final precipitate was washed with cold distilled water to remove any residual NaOH and re-dissolved in 10 volumes (v/w) with 0.5 M acetic acid. Acid extraction was continued under refrigeration (5°C) for 3 days. After 72 h the acid-soluble collagen (ASC) was collected by centrifugation (10,000*g* for 20 min at 5°C) and washed with distilled water. To remove the excess acid, the collected solution containing the soluble collagen fraction was dialyzed (molecular weight cutoff 14,000 kDa) against distilled water under refrigeration for four consecutive days and then lyophilized. The amount of the extracted collagen was determined gravimetrically.

2.9. Statistical Analysis. Physical and chemical parameters are presented as means ± standard deviation (st. dev.), normality was checked for the parametric values, and comparisons among means were made using one-way analysis of variance (ANOVA). After a homoscedasticity check, Student's *t*-test was used to evaluate statistical differences in ATP breakdown products between whole fish and fillets. The nonparametric χ^2 test was applied to detect statistical differences in the frequency of gap occurrence between the seasons. One-tailed Pearson correlation was conducted to describe relationships between the physical and chemical factors tested. Differences were considered significant at the $p < 0.05$ level. The SPSS Statistics Version 26 software (International Business Machines Corporation, Armonk, NY, USA) was used for the statistical analysis.

3. Results

3.1. Fillet Gaping Score Frequencies. The seasonal variations in gaping scores of gilthead seabream fillets are presented in

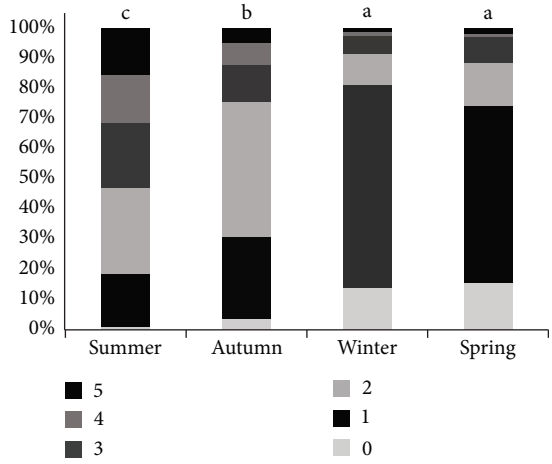

FIGURE 2: Distribution of gaping score frequencies (%) of gilthead seabream fillets in four seasons. Significant differences between seasons are indicated with different letters ($p < 0.05$). ($N = 495$).

Figure 2. Gaping score frequencies were found to be statistically different among seasons. In detail, a similar pattern was observed during winter and spring when the probability to find fillets with high gaping scores (scores 4 and 5) was small and unmarketable samples accounted only for 1% of the total, while the majority of specimens exhibited no, subtle or only mild gaping (accounting for 92% in winter and 89% in spring, respectively). On the contrary, during seasons with high water temperatures (summer and autumn, Table 1), the gaps in the muscle tissue of gilthead seabream were more pronounced. In summer, the gap in 31% of the samples was classified as severe and extreme, while the corresponding proportion in autumn was 12%. Clearly, an improvement in gaping severity was observed in autumn when compared to summer (5% and 15% of the fillets were classified as unmarketable during autumn and summer, respectively) while ~50% of the samples were found to have a gaping score of 2.

3.2. Physical Parameters. Seasonal changes in the muscle pH of whole unprocessed fish and fillets are presented in Figure 3. The pH values of the unprocessed fish were similar in the seasons with warm water temperatures (summer and autumn). The pH increased as water temperature declined (winter, spring), reaching a maximum value in spring (Figure 2). Fillets showed similar but less pronounced seasonal trends with the exception of spring, demonstrating statistically significant pH elevation compared to other seasons ($p < 0.05$) (Figure 2).

Seasonal variations in the WHC of gilthead seabream fillets are given in Figure 4. Clearly, a significant variation can be attributed to the season, as indicated by significantly higher water loss observed in summer when compared to the winter and spring samples, although individual variation among samples within each sampling point was considerable.

3.3. Proximate Composition. Seasonal changes in the chemical composition of gilthead seabream fillets are shown in Table 2. Analysis of variance indicated significant differences among seasonal samplings as regards moisture, protein, fat, and glycogen, but no significant differences were observed for ash content. The mean moisture content of the gilthead seabream fillets was higher in winter and spring, while a significant decrease was observed in summer and autumn. The mean protein content of the species remained high throughout the year. Furthermore, the summer protein values were the highest and only differed significantly from the corresponding spring values. The highest mean fat values were recorded in summer, in contrast to the winter values. Thus, fat increased in the seasons with higher water temperature. Mean glycogen content peaked in autumn and summer with values exceeding 0.5%, while the lowest value was measured in spring, thus indicating seasonality of this muscle energy component.

3.4. Chemical Freshness. No significant seasonal variation in *K*-values was detected for either unprocessed fish or fillets ($p > 0.05$; data not shown). The mean *K*-value measurements of gilthead seabream fillets and unprocessed whole fish are presented in Figure 5, while the individual nucleotides (ATP breakdown products) are shown in Table 3. Primary processing of gilthead seabream, that is, removal of scales, gutting, and filleting, accelerated ATP degradation, as denoted by the significantly lower levels of ADP and IMP, respectively, and the higher levels of ATP-breakdown end-products, namely, Ino and Hx. This caused a significant increase in *K*-values of up to 17%, thus indicating a reduction of chemical freshness due to processing, irrespective of the season.

3.5. Collagen Content and Solubility. Total collagen content and collagen types in terms of solubility, that is, acetic acid, pepsin-digested, and insoluble collagen fractions of gilthead seabream fillets are presented in Figures 6 and 7, respectively. Significant seasonal variations in collagen were evident. Fillets sampled in summer, winter, and spring contained similar amounts of collagen (4.5–5.7 mg/g), while an approximate two-fold increase in the corresponding levels was observed for fish harvested in autumn (Figure 6). During summer, ASC exhibited the highest levels, while its proportion was almost equal to the other two collagen fractions. Subsequently, ASC showed a seasonal variation as it statistically decreased in autumn, winter, and spring (Figure 7).

4. Discussion

Seasonality of gaping has been reported previously in farmed finfish species, although inconsistencies concerning this observation can be found in the respective literature. Our results demonstrating elevated gaping during warm seasons (summer/autumn) with winter mitigation (Figure 2) corroborate the patterns reported for Atlantic salmon (*Salmo salar*) by Lavety et al. [14], and Mørkøre and Rørvik [30]. However, this contrasts with studies reporting winter-peaking gaping in the same species [17, 31], and thus renders infeasible to model gaping seasonality. Based on the latter study, these discrepancies can be attributed to differences in experimental design, suggesting an interaction among gaping, growth, and season, as well as to the gaping assessment method applied

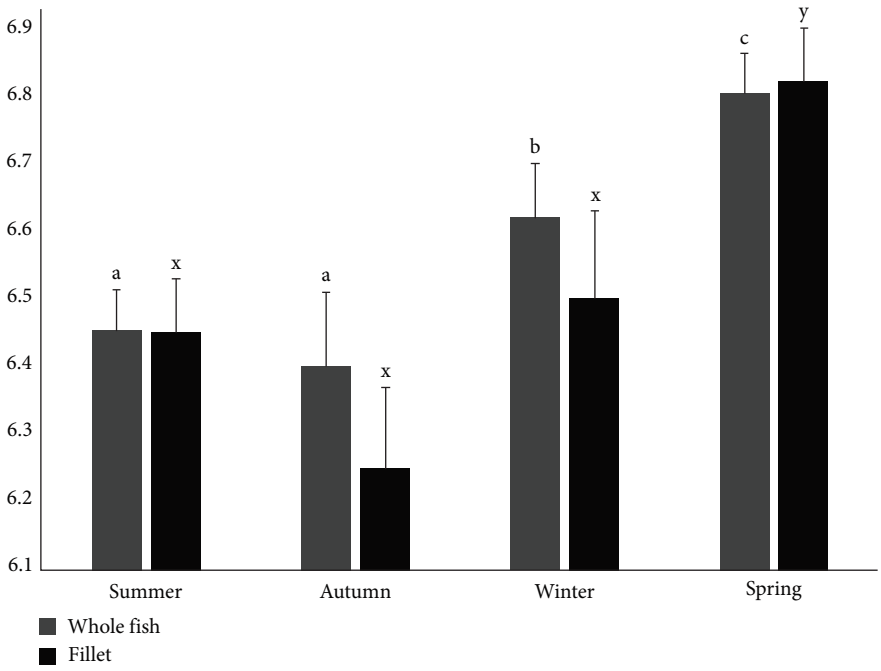

FIGURE 3: Seasonal variations in muscle pH of gilthead seabream fillets and unprocessed fish $n = 80$; fillets $n = 80$. Significant differences between seasons are indicated with different letters ($p < 0.05$).

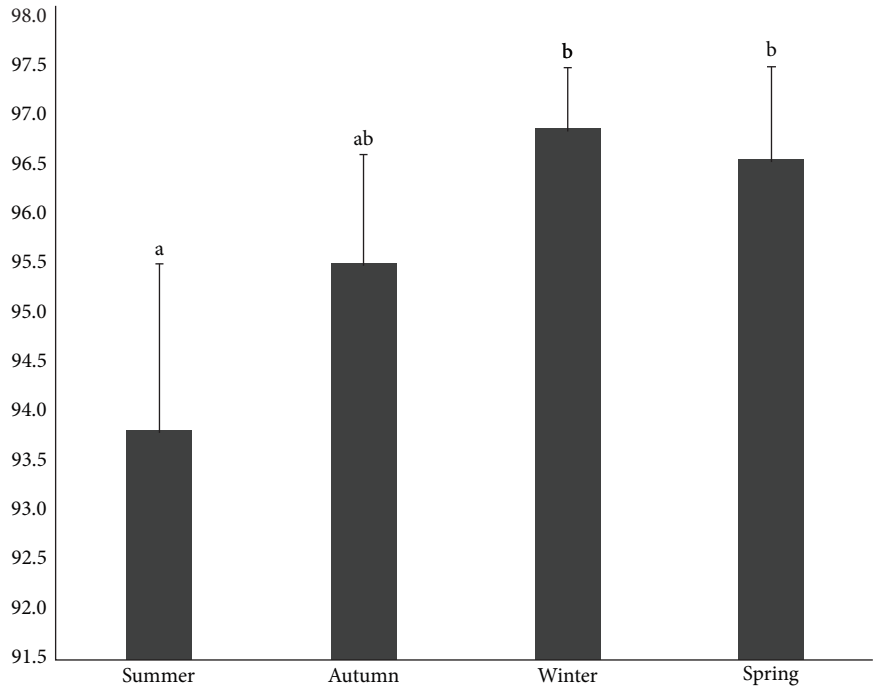

FIGURE 4: Seasonal variations in water holding capacity (WHC) of gilthead seabream fillets. Significant differences between seasons are indicated with different letters ($p < 0.05$). $n = 60$.

[31]. In our study, gaps in muscle tissue of gilthead seabream were more pronounced during summer and autumn, thus exhibiting a similar seasonal pattern with those presented in Lavety et al. [14] and Mørkøre and Rørvik [30]. Furthermore, a gaping score pattern that is similar to the one reported by Lavety et al. [14] was evident and characterized by the mitigation of gaping intensity during winter (Figure 2), when most of the specimens exhibited no, subtle or mild gaping.

Among the physical factors affecting the characteristics of muscle quality, pH and WHC have been found to be associated with gaping occurrence. In our study, seasonality

TABLE 2: Seasonal variations in proximate composition (%) of gilthead seabream fillets $n = 80$.

	Summer	Autumn	Winter	Spring
Moisture	67.9 ± 1.7^{a}	69.4 ± 1.6^{a}	73.9 ± 1.0^{b}	72.5 ± 1.3^{b}
Protein	20.9 ± 1.5^{b}	20.2 ± 0.3^{ab}	19.5 ± 0.8^{ab}	19.0 ± 1.1^{a}
Fat	9.1 ± 1.3^{c}	8.3 ± 1.8^{bc}	4.8 ± 1.3^{a}	6.9 ± 1.3^{b}
Glycogen	0.6 ± 0.2^{bc}	0.7 ± 0.2^{c}	0.4 ± 0.2^{ab}	0.2 ± 0.1^{a}
Ash	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1

Note: Significant differences between seasons are indicated with different letters ($p < 0.05$).

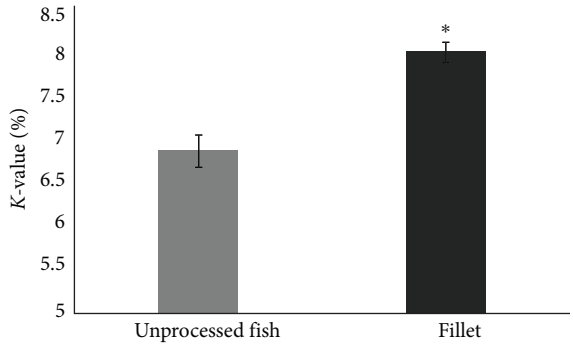

FIGURE 5: K-value measurements of gilthead seabream fillets and unprocessed fish $n = 80$; fillets $n = 80$. *Indicates the statistical difference between the tested groups ($p < 0.05$).

TABLE 3: Mean, maximum and minimum values of individual nucleotides (μmol/g) of gilthead seabream fish and fillets unprocessed fish $n = 80$; fillets $n = 80$.

	Whole fish			Fillet			*p*-Value
	Mean	Max	Min	Mean	Max	Min	
ATP	0.11 ± 0.01	0.12	0.10	0.12 ± 0.02	0.14	0.09	0.064
ADP	0.17 ± 0.03^{b}	0.21	0.14	0.15 ± 0.01^{a}	0.17	0.12	0.040
AMP	0.15 ± 0.02	0.17	0.13	0.16 ± 0.02	0.18	0.11	0.250
IMP	12.9 ± 0.86^{b}	13.8	11.4	10.5 ± 1.04^{a}	12.3	8.8	<0.001
Ino	0.89 ± 0.11^{a}	1.01	0.75	1.00 ± 0.13^{b}	1.25	0.80	0.042
Hx	0.03 ± 0.01^{a}	0.04	0.02	0.08 ± 0.03^{b}	0.15	0.04	<0.001

Note: Different letters indicate significant differences in the individual nucleotides between sample types.

was also evident for pH. The highest pH value in whole fish and fillets was recorded in spring, while a subsequent decline was observed reaching the lowest value in autumn (Figure 3). This could be due to the fact that in high water temperatures (summer, autumn), feed consumption increases, leading to higher muscle carbohydrate content (Table 2) and consequently, to lower postmortem pH, due to increased lactic acid production using glycogen as precursor [5, 14, 15, 32]. Although a negative correlation between the lowest postmortem pH and gaping has been reported in Atlantic cod (*Gadus morhua*) [32] and Atlantic salmon [14], this dependance was not evidenced by our study (correlation coefficient −0.402, $p > 0.05$). Similarly, lack of dependance between gaping and pH has been observed in several studies on Atlantic salmon [17, 31, 33]. These conflicting results suggest that pH is not an ideal parameter to monitor gaping intensity in farmed fish, despite the interaction between pH and muscle texture [31].

On the other hand, the seasonal variation in fillet WHC observed in our study (Figure 4) was strongly associated with high gaping incidence, with the correlation coefficient being −0.994 ($p = 0.003$). Although no correlation was observed between pH and WHC, it is suggested that the ability of muscle tissue to retain water molecules is affected by pH values. In detail, muscle proteins are able to retain water in their 3D structure; however, as the pH value decreases near the isoelectric point (~5.5) of muscle proteins, myofibrils shrink and protein-water interactions diminish, resulting in high water loss [33–37].

With regard to proximate composition of gilthead seabream fillets (Table 2), the reduction of fat content in winter and its redeposition, reaching the highest values during summer, is expected to be a result of the seasonal feeding pattern of the fish. Reduced feed consumption and sexual maturation are determinant factors for lower fat accumulation in the muscle tissues of gilthead seabream during winter [38]. The higher lipid deposition in muscle recorded in summer and autumn samples coincided with the increased feeding intensity of gilthead bream waxing these explanations. The seasonality in fillet moisture found in this study confirms an inverse relationship with fat [38, 39]. Carcass protein levels tend to remain stable in farmed gilthead seabream indicating the absence of seasonal variation, when adult fish of commercial weights were examined [38]. On the contrary, in our study, lower protein levels were evident in spring samples compared to their summer counterparts. Similar variations have been observed in Atlantic salmon [40], which according to the authors were related to sexual maturation. Furthermore, although no statistical significance was evident when correlating gaping with proximate composition parameters, the correlation coefficient between the values measured for gaping and fat content was 0.827 with a p value of 0.087, clearly indicating a trend. Lack of connection between fat content and either gaping or loss of fillet firmness has also been evidenced in other species [8, 19, 41, 42].

Low K-values throughout the year indicate a high freshness status, as expected in controlled commercial procedures and as required by good manufacturing practices. The high concentrations of IMP and low concentrations of the end-products (Ino and Hx) also indicate the high freshness of both intact fish and fillets. IMP and Ino concentrations in whole fish and fillets are comparable to those found for the same species, for fish stored for 0 and 3 days at 4°C, respectively [43]. In this study, it was evident that the filleting process promoted ATP-degradation cascade, as indicated by the significantly higher *K*-values observed in fillet tissues when compared to those of the whole fish. This is probably related to upregulation of the endogenous enzymes involved in the catabolic pathways of the ATP molecule due to increase of body temperature and tissue mechanical damage during scale removal and filleting. The absence of seasonal variations in the *K*-values suggests that the gaping

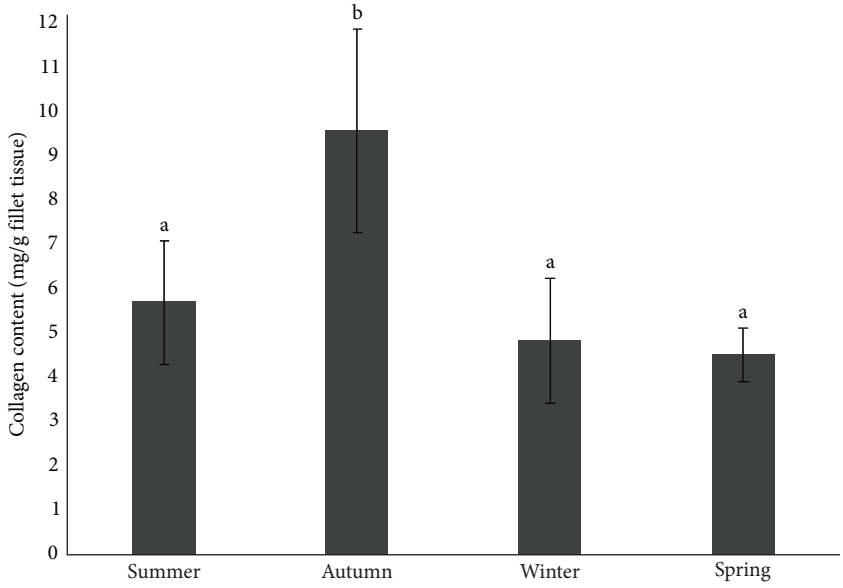

FIGURE 6: Seasonal changes in total collagen content (mg/g) of gilthead seabream. Significant differences between seasons are indicated with different letters ($p < 0.05$). $n = 40$.

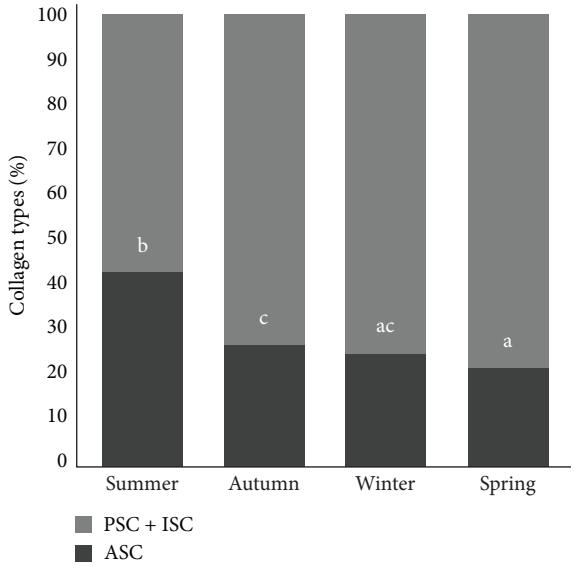

FIGURE 7: Seasonal changes in muscle collagen content (%) expressed as acid-soluble collagen fraction (ASC) and pepsin-soluble and insoluble-collagen fraction (PSC + ISC). Significant differences between seasons are indicated with different letters ($p < 0.05$). $n = 40$.

phenomenon is not related to the aforementioned index when the fish are handled in a similar manner (slaughter procedures, storage time prior to processing, etc.). Gaping intensity, on the other hand, has been associated with storage time prior to filleting, that is, pre- or postrigor fillet production. Specifically, more breaks in the myocommata have been reported in postrigor produced cod and salmon fillets compared to their prerigor filleting counterparts [5, 15, 44, 45]. These observations have been attributed to the avoidance of tension amplification in muscle structural components, given that muscle fibers contract during the rigor mortis process [5], and to the low postrigor pH associated with decreased connective tissue strength [46]. It should be emphasized, that there is also a connection between the progress of rigor mortis and ATP depletion [47, 48]. In a study by [49] on blue tilapia (*Areochromis aureus*), in particular, it was evident that the ATP/IMP ratio declined with the progress of rigor mortis, indicating a strong link between these two parameters. Although the development of rigor mortis was not evaluated in this study, very low ratios of the aforementioned nucleotides were found at all sampling points, thus supporting the hypothesis that the samples were at the same stage of rigor mortis. However, this assumption needs to be verified because the ATP breakdown pattern varies among different fish species [50].

This study also includes the first attempt to evaluate the effect of season on the collagen content of gilthead seabream muscle tissue. The total collagen value was similar in summer, winter, and spring, while a two-fold increase was evident in autumn. Similarly, seasonal variations in collagen have been reported for the greater amberjack (*Seriola dumerili*), with the higher values found during autumn and the lower ones during winter and spring [51]. On the contrary, no significant differences in annual collagen levels have been found for red seabream (*Pagrus major*) [52], albeit belonging to the same family as the species of our study (Sparidae). In the aforementioned study, however, extremely low levels of collagen were found (~0.8–1.4 mg/g), compared to those reported for other fish species, including gilthead seabream [53–56]. Touhata et al. [52] associated these discrepancies with sample collection, where the collagen-rich myocommata [57] were eliminated. Our results are similar to those of Suárez et al. [55] who reported collagen levels in gilthead seabream ranging from 4.6 to 5.8 mg/g during ice storage for 2–120 h, although the sampling season was not mentioned.

In general, collagen levels reported in the literature range from 3.4 to 21.9 mg/g in different fish species [58, 59], indicating considerable species-dependent variations. A connection between gaping and collagen content has been reported, suggesting that those species with collagen levels of 3.4–5.1 mg/g display a greater propensity to gap [53, 58–61]. In our study, the correlation between gaping and total collagen was 0.204 ($p > 0.05$), indicating no link between them. Indeed, it has been confirmed that the amount of total collagen is not the pivotal factor for gaping occurrence, and the link is influenced more by the properties and composition of collagen in the extracellular matrix [17, 62, 63]. Based on these observations, collagen solubility was also evaluated. A significant effect of season on collagen types was found, in terms of collagen solubility, as indicated by the high levels of ASC in summer, with a subsequent reduction in autumn, winter, and spring (Figure 7). In line with our summer findings, ASC has been reported to be the most abundant collagen fraction in several fish species [64–67]. Aidos et al. [60] reported 33.8% ASC in Atlantic salmon in January, which is similar to the winter measurements of our study. Similarly, 31.8% soluble to acetic acid collagen was found in the same species in February, while the corresponding amount was much lower (17.7%) in June, when the gaping phenomenon was more pronounced [17]. In gilthead seabream, the reported value of ASC 2 h postslaughter was close to 17% [55]. The connection between the gaping phenomenon and ASC observed in this study appeared to be significant, with a correlation coefficient of 0.975 ($p = 0.013$). Espe et al. [17] found higher levels of soluble collagen in Atlantic salmon during winter when compared to summer, which coincided with more gaping incidences. A higher percentage of soluble collagen has also been linked with softer muscle tissue [57, 68], clearly indicating that collagen fractions are closely related to the gaping phenomenon.

5. Conclusions

Overall, our study demonstrates the relevance of the factors affecting the fillet integrity of farmed gilthead seabream when examining the seasonal variability of physical and chemical parameters. Moreover, it proves that the harvest season affects both the muscle characteristics and the gaping severity in this species. Gaps in muscle tissue of gilthead seabream peaked during summer and autumn, coinciding with lower pH and WHC values. These observations are probably related to the high feeding intensity during periods when Mediterranean farmed fish exhibit high metabolic rates. No significant correlations were found between gaping and proximate composition or total collagen content. However, a strong connection between gaping incidents and collagen fractions is evident. Based on the higher amount of ASC observed during summer, it can be concluded that collagen solubility affects gaping in farmed gilthead seabream. Further research is required to fully comprehend the connection between variations in collagen composition and failure to hold the connective tissue together in farmed gilthead seabream.

Data Availability Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

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